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# Cellular xenotransplantation of animal cells into people: benefits and risk

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**Summary**

The main benefit of xenotransplantation is its potential to overcome the worldwide organ shortage experienced in allotransplantation. Allogeneic transplantation is the only successful therapy for several life-threatening diseases, with cell, tissue or organ donation only partially meeting the demand and many patients dying while waiting for treatment. With supply falling short of demand, it is forseen that the use of porcine material may at some stage overcome the existing gap between organ availability and clinical need. Recently, pig islet cells have been utilised in clinical trials, with safety being demonstrated. Indeed, pig-derived cells present several advantages: *i)* porcine cells have a stable function and differentiation pattern and are not tumorigenic; *ii)* pig cells have been shown to meet the

physiological needs in large animal models; *iii)* the source of pig cells can be scaled-up to meet demands in a highly standardised manner, and with respect to animal welfare regulations; *iv)* designated-pathogen-free (DPF) pig lines can be produced, which could result in a higher safety profile than allotransplantation itself; *v)* the risk of zoonosis, which was raised years ago as the major hurdle, has been recently circumvented and is actually viewed as a controlled risk; and *vi)* immune risks are being circumvented via the use of genetically modified donor animals and encapsulation of porcine cells, particularly for the treatment of diabetes. Overall, the benefit appears to outweigh potential risks with respect to cellular xenotransplantation and this is discussed further in this review.

**Keywords**

Cells – Organs – Porcine – Virus– Xenotransplantation.

## Background

Transplantation represents the ideal treatment option for many patients in terminal organ failure. Indeed, it has been clearly demonstrated that transplantation is associated with improved quality of life, extended patient survival, and reduced costs to society. However, transplantation is severely limited by a tremendous organ shortage and, as a consequence, benefits only a minority of patients. Most patients must continue with ongoing, expensive treatment. For instance, worldwide, over two million people affected by chronic kidney disease currently receive treatment with dialysis (instead of a transplant) to stay alive, with an overall cost of around 80 billion euros per year. Likewise, it is estimated that the total worldwide number of type 1 diabetic patients regularly injecting insulin is between 10 and 20 million (1).

In light of this, it is of the utmost importance to identify novel sources of organs, tissues or cells to satisfy the clinical need. In this context, xenotransplantation, or transplantation of organs, cells or tissues between individuals belonging to two different species, such as from animals into humans, represents a potential solution to meet this

critical need. Although the immediate benefits are clear, this line of biomedical research is increasingly reliant on the use of large animals, notably the pig, which raises a number of associated risks. A full assessment of these benefits and risks is required for the development and clinical application of xenotransplantation for the purpose of improving human health. Ethical requirements are high on the list of importance of scientific societies, policy-makers and international agencies such as the World Health Organization and are heavily embedded in the regulatory frameworks dealing with xenotransplantation (2, 3, 4). Indeed, ethical considerations are an integral part of any xenotransplantation practice, and experts in ethics have been, and still are, involved in these important issues and are contributing to the development and harmonisation of guideline policies (5). This, however, is not the purpose of this paper and will not be discussed further.

## Assessing benefits and risks

### The risk of infection

Each innovative medicinal product carries its own risks when administered to a patient, and a xenotransplantation product is no exception. The first risk factor that attracted attention was the potential transmission of infectious agents to the recipient, with resulting disease. This is relevant in view of the fact that a xenotransplantation product comprises living xenogeneic cells/tissues/organs, which precludes the sterilisation procedure that is possible for drugs and biologicals. The microbial risk of xenotransplantation is similar to that of autologous and allogenic cell therapy products; however, due to the nature of the source material, i.e. non-human, it is deemed more complex, as it is not known how certain animal pathogens will respond in a human host environment. Among the first documents from regulatory agencies addressing this item was the Public Health Service Guideline on Infectious Disease Issues in Xenotransplantation issued in the United States in 2001 (6). Subsequently, the Guidance on Xenotransplantation issued by the Food and Drug Administration (in 2003 and recently updated in 2016)

(7), and the Guideline on Xenogeneic Cell-based Medicinal Products issued by the European Medicines Agency (EMA) in 2009 (8) have provided more details. These regulatory documents address products from any species transplanted to humans, but in the following discussion, the donor will be limited to the swine species because this is currently the generally accepted species for a xenotransplantation product.

The microbial risks of a xenotransplantation product can roughly be divided into three main categories (7):

– transmission of infectious agents that are pathogenic for humans but may not be pathogenic or even detectable in the source animal host

– transmission of organisms that may not normally be pathogenic in humans but can become so in the immunosuppressed or immunocompromised individual

– recombination or re-assortment of infectious agents, particularly viruses, with non-pathogenic or endogenous human infectious agents, to form new pathogenic entities.

These categories, however, do not mention infectious agents that are pathogenic for swine and can cause swine disease.

Concerns about the microbial safety risk of using pigs in xenotransplantion were raised when there were reports of pig-to-human transmission of porcine endogenous retrovirus (PERV) (it should be emphasised that clinical trials at that time were not discontinued, but only put on hold awaiting the development and implementation of monitoring protocols) (9). Subsequently, the initial concerns about the human tropism of PERV developed into a more general concern about a wider range of pathogens, including exogenous infectious agents, in particular, porcine viruses. This concern about transmission of infectious agents from donor to human recipients has resulted in the use of a new designation, namely ‘designated-pathogen-free’ (DPF). (It is important to note that DPF does not mean that animals are gnotobiotic, i.e. devoid of any

infectious pathogen in all body compartments, including the alimentary tract; such a status is even more complicated to achieve in sustained production than the DPF status. Also, endogenous pathogens such as PERV are not included in the DPF status simply because of their endogenous presence.)

Like the designation 'specific-pathogen-free' (SPF), which is widely used in biomedicine, DPF designation is not associated with a single prescribed list of pathogens that should not be present in the donor animals. Instead, pathogen exclusion lists, which should be presented and agreed with regulatory agencies, are proposed by the regulator, scientific community (10) and also by institutions preparing for clinical trials (11, 12). These lists may differ between continents and between countries, depending on the infectious agents that are present. There may also be different lists required for different xenotransplantation products, depending on the organ and tissue distribution of infectious agents and the processing of the organs or tissues before being administered to patients (13).

Achieving the biosecurity barrier needed to achieve DPF status requires special conditions of breeding and husbandry (7, 14, 15). The most relevant factors to consider in maintaining a barrier to disease and preserving the health status of the animals are the building, the location, feed, staff, and the number of animals. The number of animals within the biosecure barrier should not only be sufficient for production of donors in, for example, clinical trials, but also be sufficient to avoid inbreeding in outbred animal herds. In general, the operations in the animal facility should be in compliance with the Good Manufacturing Practices employed in similar fields. Also, accreditation by the Association for Assessment and Accreditation of Laboratory Animal Care (www.aaalac.org) is recommended.

From a risk perspective, there should be a programme for regular monitoring of the infectious agents on the agreed list of pathogens to be excluded. The frequency of sampling (blood, secretions or faeces) from the donor animals or of detailed investigation of sentinel animals, depends on the health status of the herd after its initial

population and the emergence and management of infection or disease outbreaks. Under high-hygiene biosecure conditions, DPF status can usually be achieved without the need for vaccination, and the use of antibiotics can be avoided.

It is evident from the above that meeting the requirements for DPF status translates into a costly enterprise. In the US context, this could easily increase the price fifty-fold for one individual young-adult pig (six months old), which makes it impossible to produce for high-volume administration. However, the basic question of whether or not there is indeed the need for expensive biosecure facilities for donor animals for a xenotransplantation product, i.e. medical-grade pigs, remains unanswered. Similarly, it is unclear whether the long storage periods recommended in regulatory documents governing sampling and the archiving of samples from the donor and recipient are justified. These documents recommend storage in a deep-frozen state for a long time, i.e. 30–50 years, but there is no rationale given for this instruction, except for one statement in the Public Health Service Guideline in the United States, which indicates that the requirement for long storage periods is based on '*the latency periods of known human pathogenic persistent viruses and the precedents established by the US Occupational Safety and Health Administration with respect to record-keeping requirements*' (2). This indicates that the requirement for archiving is essentially a public health issue. It should, therefore, receive attention from not only the sponsor of clinical trials and the institution marketing the product, but also the governmental institutions overseeing public health. The issue falls outside the remit of the usual interactions between these parties and should be a separate item of discussion. This discussion should also address aspects such as the public/private sponsoring of archiving, ownership of the archive, and access to the archive.

It is clear that it is important to discuss the requirements for archiving and, as stated above, it is equally important to question whether a barrier facility is really necessary for all types of xenotransplantion. Not having to maintain a biosecure barrier would significantly reduce

the cost risks. The following points are worth noting when considering whether or not such a barrier is necessary:

– Caesarian sectioning and colostrum deprivation of the first population in an SPF-like facility may be sufficient (14). In other words, the building and operations are under less strict conditions than they would be if DPF status were required. An example is the facility for minipigs run by Ellegaard-Göttingen in Denmark (16, 17).

– A proper selection of founder animals in facilities with high hygiene standards could serve the same purpose. A requirement for such facilities is that they be siutated in remote locations so as to avoid potential entry of pathogens from the environment.

– Barriers other than physical barriers could serve to establish a DPF status. An example is the placenta, in the case that newborn piglets are used as the donor. In this case, the sows should be monitored during pregnancy for pathogens and virus activation, which can result in transmission through the placenta.

– To assist with the costs associated with barrier facilities, some xenotransplantation products could be subject to a restricted pathogen exclusion list if, for example, distinct cells or tissues lack infection with specific viruses of concern. Cell products that are encapsulated before implantation could also be subject to a restricted list, as discussed in the recently published update of the consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes (8, 18).

– The processing of tissue and organs and the time period of processing should also be considered. This particularly applies to some cell therapy products that require a long period of culture after cell isolation or establishment of cell lines before the product is released for administration. In this situation the microbial presence in the pig is quite remote from the final product (11, 13).

It thus appears that a high-hygiene biosecure barrier might not be necessary in all cases of a xenotransplantation product. Hence, instead

of concentrating on intense husbandry conditions and animal herd monitoring, the focus could shift to detailed pathogen screening of the product before it is released. In this discussion, it should be noted that there is almost no information on how the microbial status of a donor relates to pathogen transmission and the development of disease in the host, although we do have some recent work demonstrating a lack of correlation betweeen the donor and tissue microbial status (19). It has been proposed that some exogenous pathogens that can be transmitted by pigs, such as hepatitis E and viruses in the herpes family (e.g. lymphotropic herpes viruses and cytomegalovirus) do pose a xenozoonotic risk (20), but the basic question of whether or not a xenotransplantaion product poses an infectious risk has not been answered definitively. At the moment, the assessment of microbial safety is mostly limited to animal models used for the purposes of testing the efficacy of xenotransplantation products, which are not always suitable.

It is quite understandable that, considering the many unknowns regarding potential infection of a recipient of a xenotransplantation product by the product itself, regulatory agencies, in a risk-aversive approach, require donor animals to be devoid of any infectious pathogen. But studies, as well as many trials with/without regulatory oversight on living porcine products administered to humans, have, to date, not shown indications for pig-to-human pathogen transmission. Essentially, all the studies conducted to date suggest that a porcine xenotransplantation product is well tolerated and fairly safe, including from a microbial standpoint (12, 21, 22, 23). It has also been noted in the literature that the risk from PERV may no longer be as significant as initially perceived (24, 25, 26).

Thus, it is tempting to conclude that, with the suggested strategies in place, xenotransplantation products will have a much better pathogen safety profile than the allogeneic living organs, tissue and cell preparations that are currently used in clinical practice. Selection of donors meeting high-quality health standards, and implementation of rigorous quality control to monitor organ and ischemia damage during procurement, transport and processing, are of high importance. So, at

least in theory, the ultimate risk–benefit ratio of a xenotransplantation product is expected to be much higher than that of a human-derived allogeneic product.

At this point, it is worth providing further explanation of the word 'risk'. In many operations and industrial product manufacturing, and in regulatory documents, this word receives detailed attention. It is not only a question of 'risk', but of managing the risk. The regulatory agencies of the European Union, Japan and the United States recommend the adoption of the Quality Risk Management (QRM) guideline issued in 2005 by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (27). Essentially, the 'risk' approach is separated into various steps in a continuous process which includes risk assessment (identification, analysis, and evaluation), followed by risk control (reduction and acceptance), and finally risk review. Both the evaluation of individual infectious agents and the evaluation of the product manufacturing process are expected to not only facilitate the discussion between sponsors and regulatory agencies, but also to facilitate the development of xenotransplantation products by improving the process of transforming research material into a product for clinical administration.

## Risks posed by clinical applications

Cell replacement therapies represent innovative alternative treatments offering the promise of long-lasting restoration or amelioration of disrupted cellular functions rather than temporary alleviation of clinical symptoms necessitating lifelong medication. Patients suffering from degenerative and auto-immune diseases could benefit from such treatments. Pancreatic islet allotransplantation for the treatment of type I diabetes is a clinical reality, the transplantation of other cell types, such as hepatocytes, bone marrow or umbilical cord stem cells, is also a reality in the clinic today, and neural cell transplantation is in the early development stage (28, 29, 30). However, and regardless of tissue-specific considerations and difficulties, a common major obstacle standing in the way of widespread use of cell transplantation

is the lack of human donors, and this has led to the search for alternative sources of cells from other species. In this context, pigs have emerged as suitable candidates for providing xenocells due to anatomical and physiological similarities with humans. In the case of treatment for type I diabetes, porcine islets could meet the demand and have several advantages, including the functionality of porcine insulin in humans due to high similarity in protein sequence, which supports the case for using pigs as a donor species (reviewed in [31, 32]). Moreover, it is possible to genetically modify donor pigs to mitigate the host immune reaction to xenografted cells and to adapt their function to human physiology when needed, which will definitely accelerate the transition of cell xenotransplantation from the bench to the clinic. The first exploratory health economic evaluation of a porcine islet xenogenic cell therapy product showed that this product may prove to be a cost-effective and possibly cost-saving procedure for type 1 diabetes compared to standard management using insulin treatment, even thought the costs per pig are quite high (33).

Regarding methods of delivering islets into the recipient, the methods currently preferred are: intra-portal transplantation, as performed in allotransplantation, for free porcine islets (wild-type or genetically modified); extra-peritoneal implantation for macroencapsulated islets; intraperitoneal implantation for microencapsulated islets; and subcutaneous implantation for macroencapsulation devices. Intramuscular implantation of free islets is also currently under investigation (28, 34, 35, 36). However, the free transplantation of such non-encapsulated cell sources, in humans, is not yet permitted by the EMA due to possible transfer of pathogens and because of the risk of uncontrolled cell replication in the recipient organism, although a recent study demonstrated a lack of pathogen transmission to marmosets (23).

Another limitation of cell transplantation is the need for immunosuppression. Allo- or xeno-transplantation of insulin-secreting cells in humans is possible, but it elicits a severe immune rejection requiring immunosuppressive treatment of the recipient. This treatment, although beneficial in preventing islet rejection, has

significant side effects, such as diminishing islet function or induction of islet death, kidney toxicity and diabetogenicity. Immune isolation of cells by encapsulation is a promising strategy for both allogenic and xenogeneic cell transplantation. This avoids lifelong immunosuppression and should be readily implemented in the clinic. Permselective membranes protect the transplanted cells against the recipient's immune system, but allow oxygen and nutrient supply (reviewed in [37]). There are still major immunological obstacles to successful clinical islet xenotransplantation, but strategies to overcome these have been developed. Some genetic modifications, e.g. elimination of proinflammatory molecules or insertion of transgenes to improve survival and function, may also be beneficial for encapsulated islets (31). Alternatively, human beta-cells can be produced from pluripotent stem cells. Their delivery route strategy is similar to that described above, as avoidance of immune rejection of allogenic donor cells follows the same logic. In theory, use of patient-derived induced pluripotent stem cells (iPSCs) would allow for autologous beta-cell production and transplantation. However, this is considered an economically non-viable option at the moment due to the current technological limitations.

A number of clinical xenotransplantation trials in humans have already been described in the literature and deemed safe (12, 13, 22). Efficacy confirmation requires further research; however, in the context of islet cells, the risk is considered low.

## Risks posed by genetic modification

Cutting edge research is dependent on genetic engineering/genome editing for the purpose of creating suitable surrogates for the study of genetic diseases (38, 39, 40, 41) and regenerative medicine (42), or for supplying an unlimited source of cells, tissues, organs and scaffolds that can be used effectively and safely for transplantation to cure human diseases (43). Multi-transgenic pigs have already been generated by classical means of homologous recombination and/or random integration. The development of GGTA1-KO pigs was a fundamental step in reducing the risk of immune rejection in

xenotransplantation. Now, multiple transgenes can be introduced on a donor genetic background, not only to mitigate immune complications but also to improve the function and production of insulin (44). With the use of programmable nucleases, multiple recombination events (ins/del or homology directed repair) can be obtained in one single step. Targeted integration can ensure the optimal performance of any transgene integrated, therefore increasing the efficacy of the expected function.

To date, the multi-transgenic pigs generated by conventional means for xenotransplantation are healthy and can reproduce. However, unexpected phenotypes can develop after repeated rounds of multiple genetic engineering, vital function can be affected, and side effects due to inadequate transgene function can occur. With genome editing, the same concerns may arise, but the precise genetic modification that can be obtained should be of less concern. Genome editing technology has significantly evolved since the initial reports (43). Accordingly, recent work has demonstrated remarkable survival rates. For example, in baboons, the use of transgenic pigs for heterotopic cardiac xenografts, coupled with an aggressive immunosuppression regimen, resulted in post-transplantation survival rates of up to almost three years (45).

Progress in the field of xenotransplantation has changed gear since nuclease-based techniques have been implemented for editing the pig genome (46, 47, 48, 49). The number of transgenic animals with inactivated genes and/or integrated novel transgenes has dramatically increased, thus facilitating the development and completion of pre-clinical studies (40, 41, 42, 50, 51, 52, 53, 54). Genome editing in live pigs is required to test the safety and efficacy of this technology for xenotransplantation products. Although potential off-target effects are often indicated as possible complications, animals generated after genome editing do not appear to be different from those generated by conventional technologies. Therefore, there are no fundamental reasons why genetically modified pigs utilising gene editing pose *a priori* different risks compared with those engineered by conventional

transgenesis. However, risks should be carefully assessed case by case depending on the modified/added genes.

Finally, PERV has been the main focus of concern in gene editing due to their presence as integrated elements in the pig genome, but recent work using CRISPR/Cas9 technology has convincingly demonstrated that multiple copies of PERV can be targeted simultaneously to obtain PERV-free cells (55). However, it is yet to be demonstrated that this technology can give rise to healthy animals.

## Conclusion

It is clear that the use of xenotransplantation to treat human disease would be of great benefit but can pose a risk. However, it is also clear that many of these risks can be overcome with careful consideration and planning. The future of clinical xenotransplantation is particularly bright with respect to the use of porcine cells and their use should be supported as an alternative to current methods.

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